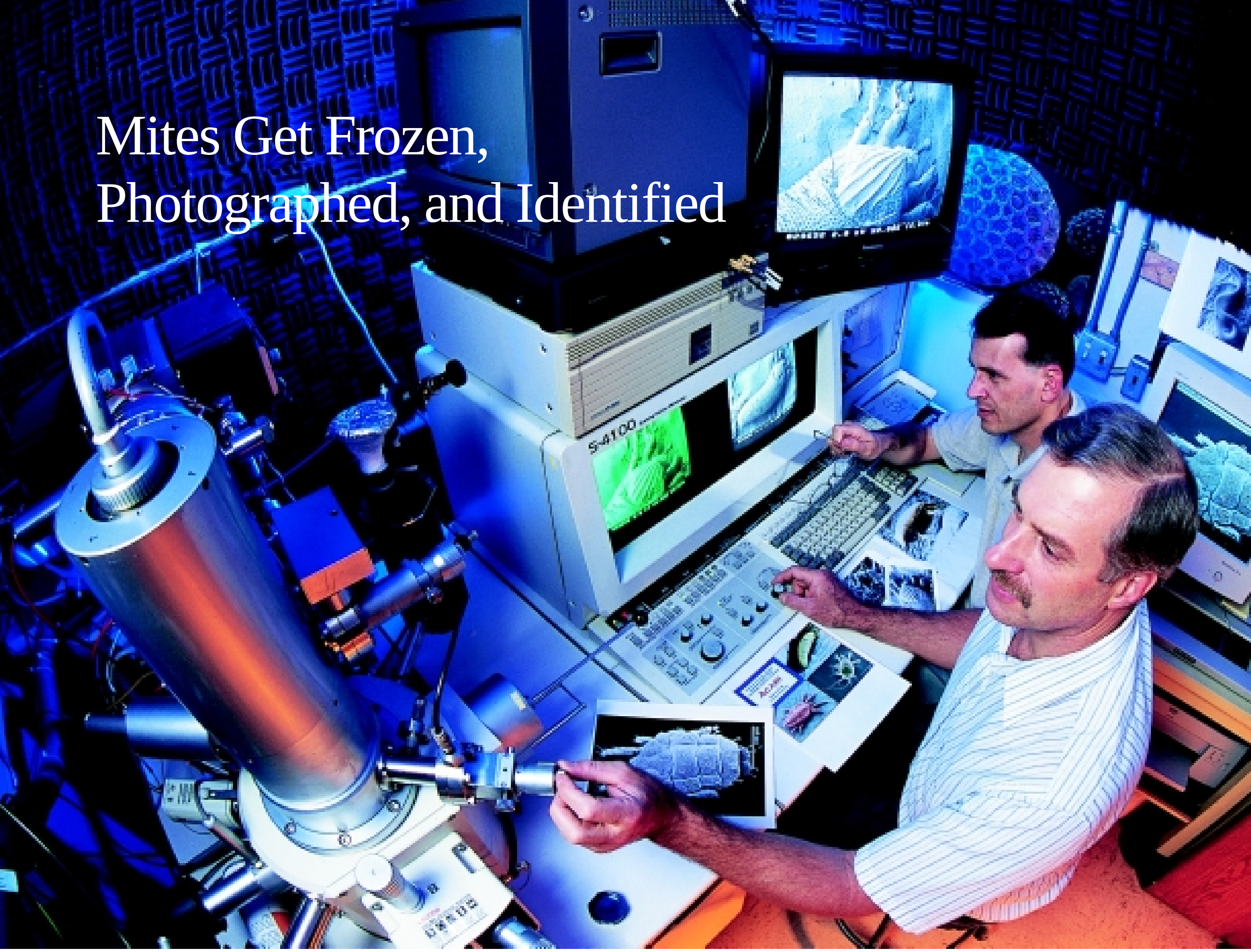
Mites Get Frozen, Photographed, and Identified



SCOTT BAUER (K9077-1)

K9077-21

Scanning electron microscopy allows mites to be viewed from different angles. This is an overhead view of the *Lorryia formosa* mite shown on the cover. Magnified about 200x.

K9077-26

A straw itch mite *(Pyemotes tritici)* on the back of a caterpillar. A major problem in stored grain, this mite is also a potential biocontrol agent. Magnified about 375x.

K9077-27

The dust mite *(Tyrophagus putrescentiae)* is common on plant leaves and in stored grain and animal feed. Magnified about 100x.

Flash-freezing under high magnification allows improved study of mites

Ronald Ochoa, the U.S. Department of Agriculture's chief expert on mites, is about to transform the 200-year-old study of mites—the science called acarology. Ochoa is a research associate at the Agricultural Research Service's Systematic Entomology Laboratory (SEL) in Beltsville, Maryland. He specializes in the systematics of mites—that is, the discovery, scientific description, classification, and naming of agriculturally important mite species. He is also curator of mites for the National Collection of Insects and Mites, housed at the SEL. The collection belongs to the Smithsonian Institution's National Museum of Natural History in Washington, D.C.

A low-temperature scanning electron microscope (at left) enables acarologist Ron Ochoa (background) and botanist Eric Erbe to observe tiny mites in detail impossible with conventional microscopes and slide-mounting techniques. Liquid nitrogen is first used to flash-freeze mites on their hosts in their natural positions.

Recently, Ochoa teamed up with cytologist William P. Wergin and botanist Eric F. Erbe, both of ARS' Beltsville Agricultural Research Center Nematology Laboratory, to study mites by using newly developed technology called low-temperature-scanning electron microscopy (LT-SEM).

"Mites have attacked the world's vertebrates, invertebrates, and plants for millions of years," Ochoa says. "And although they remain a constant threat to economically important crops, stored grains, livestock, wildlife, and humans, only about 10 percent have been described or named."

Mites come in a variety of body shapes and normally have four pairs of legs. "But because of their small size—some no bigger than the point of a needle (80 micrometers in diameter)—mites are difficult to study biologically," Ochoa says. "Their intricate structures and the distribution of their hairs, called setae, are important for identifying them, but their minute size can be a problem."

Lack of detailed information about mites' correct identity, biology, and

SEM mite photos by Eric Erbe; digital colorization by Chris Pooley.

K9077-25

The peacock mite *(Tuckerella* sp.*)*, a beautiful but important pest on citrus in the Tropics, is shown on tea stem. Magnified about 65x.

K9077-23

The rust mite *(Aceria anthocoptes)*, here on Canada thistle, may have potential as a biological control agent of this weed. Magnified about 700x.

K9077-24

The flat mite *(Brevipalpus phoenicis)* carries the leprosis virus in citrus, a disease currently in South America but moving north. Magnified about 150x.

ecology often causes serious consequences to U.S. agriculture. "More than 6,000 mite species infest nearly every plant important to agriculture," Ochoa says. "In the United States, they cause annual economic losses estimated in the billions of dollars from decreased food, fiber, and ornamental production. Invasive mite species are one of the primary culprits."

Increased world trade will continue to distribute mite infestations widely. "Once they've become established in a new area, certain biological characteristics allow mite populations to escalate rapidly to pest status," Ochoa says. "High egg production, various modes of reproduction, short life cycles, many dispersal techniques, and adaptability to diverse environmental and ecological conditions all contribute to their success."

Magnifying the Minuscule

Ochoa says that LT-SEM technology—developed by Wergin—provides a powerful new tool that can benefit not only the systematics of mites, but also research on other microorganisms.

"Unlike conventional microscopes," Wergin explains, "an SEM apparatus does not use light passed through a glass lens to magnify images of a specimen. Rather, the images are formed and magnified by electrons passing through a magnetic field that functions as a lens. The images can be displayed and recorded on a cathode ray tube similar to one in a television."

Says Ochoa, "LT-SEM was used to obtain—for the first time ever—highly magnified, clear images that show the details of intact mites and how they interact with and attack plant and insect hosts."

To prepare, or fix, a mite specimen for viewing, Ochoa and Erbe use liquid nitrogen, which is at –320˚F. This cryofixation instantly freezes the mite in its natural state on the host and prevents it from moving or becoming distorted.

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The bee-infesting *Varroa* mite causes considerable economic losses for beekeepers and agriculture. Cytologist Bill Wergin (left) and entomologist Jeff Pettis examine a highly magnified photo of a honey bee infested with *Varroa*.

"Although conventional SEMs have been used by acarologists for the past 30 years, Wergin's technique has many advantages," says Ochoa. "We can magnify mites more than 50,000 times. For the first time, we can observe very delicate structural details at different angles and can see mites in three dimensions, fully hydrated, and on their hosts in natural positions. Using LT-SEM will help us determine how these arthropods perceive and react to their environment and how they're affected by factors like light, temperature, moisture, and pressure."

Anatomical Answers

According to Ochoa, one major problem in understanding and managing mite pests has been scientists' inability to observe the relative position and function of organs in a slide-mounted mite.

"Traditional slide-mounting procedures flatten or distort specimens, which seriously limited our ability to accurately see the relationships between mites and their hosts," says Ochoa. "Recent studies show that the chemicals used to fix specimens may also cause physical changes that obscure important distinguishing characters, like setae, used to identify species.

"Mites have many setae, sensory organs, mouth parts, and other body parts that are so complex that systematists have difficulty comparing or contrasting those of closely related species," says Ochoa. "Many external characteristics used for identifying flat, rust, gall, and bud mites are related to their body ornamentation."

LT-SEM reveals ultrastructural features—like pores—on the surface of mites' bodies that have never been seen before and that provide vital information on how they move and hold onto their hosts. Since many plant-feeding mites are host specific, such information could be very valuable.

"All these new data will increase our knowledge of the plant-feeding mites already in the United States and of those being introduced," says Ochoa. "It will help determine the potential destructive nature of new invasive species and help scientists and quarantine officials develop better management, control, and quarantine methods."

So far, Ochoa—in cooperation with entomologist Carl C. Childers at the University of Florida-Lake Alfred and acarologist Barry M. OConnor at the University of Michigan-Ann Arbor—has used the new technology to study several mites on plants. He's also used the LT-SEM technique to compare different traditional slide-mounting methods, examining several plant-feeding species of eriophyid and flat mites.

"Today, there are 3,400 described species of gall, rust, and bud mites," Ochoa says. "This is just a small fraction—perhaps 5 percent—of the total number estimated to exist."

Many of these species can spread destructive viral, microbial, and fungal diseases. For example, a flat mite can carry the citrus leprosis virus—a very important and costly plant disease in South America.

According to Wergin and Ochoa, one important scientific observation and theory of mites has already come from using this new technique: the discovery

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The mite species *Aceria anthocoptes* may prove useful as a biological control agent for weeds. From left to right, Chris Frye, botanist with the Maryland Department of Natural Resources, ARS acarologist Ron Ochoa, and ARS plant physiologist John Lydon identify a thistle species as *Cirsium horridulum* and note where the highest mite population is most likely to be.

that some mites' setae appear to function as capacitors—possibly helping the mites sense electrical or magnetic fields in their environment.

"These sensing devices may help them find food or avoid predators," says Ochoa. If the theory is true, perhaps artificial fields could be generated to confuse or disorient the mites and reduce the damage they cause to people and agriculture. This discovery could help scientists develop safer and more effective methods of controlling these pests," he says.

To Curb a Weed

Ochoa is also working with ARS plant physiologist John Lydon at the Weed Science Laboratory in Beltsville. They are studying a mite as a possible biological agent to control Canada thistle *(Cirsium arvense)*, a major invasive weed pest in U.S. pastures. First identified in Europe over 100 years ago, this mite *(Aceria anthocoptes)* was discovered in the United States in 1998, when Lydon collected some Canada thistle on Maryland's Eastern Shore.

"Preliminary results from a survey of Maryland and surrounding states indicate that the mite is abundant here," Lydon says. "Under growth-chamber conditions, mite populations on a Canada thistle plant can reach very high levels, causing severe damage to the plant."

Their presence leads to a reddish-brown discoloration of thistle leaves, leaf curling, and spindly growth. Says Ochoa, "These mites could also transmit plant diseases—particularly viral diseases—to the weed. The LT-SEM will allow us to measure the number of mites per leaf and see their damage to leaves."

Lydon wants to determine the size of the mite population needed to significantly impede growth of Canada thistle and whether this mite can transmit a disease or a virus to the weed. A search is under way for viral-infected Canada thistle plants in the areas where they were once reported—Denmark, England, and North Dakota. Lydon also plans to determine the specificity of *A. anthocoptes*—whether it attacks other *Cirsium* species.

Helping Bees With *Varroa*

Ochoa is working with ARS entomologist Jeffery S. Pettis at the Beltsville Bee Research Laboratory to see if the LT-SEM technique may help bee researchers better understand how parasitic mites like *Varroa* interact with their bee hosts.

"*Varroa* mites feed on the blood of adult and developing bees," says Pettis. "Parasitized bees may have deformed wings and abdomens and a shorter life span than unparasitized hivemates."

Because the LT-SEM freezes and captures *Varroa* mites on bees at the moment of parasitization, the scientists have discovered some intriguing behavioral and structural patterns that allow the mite to hide on the bee.

"*Varroa* mites may camouflage themselves by aligning their setae with the hairs on the bee's body. In doing so, they could escape detection by the bee when it grooms itself or when it's being groomed by another bee," says Pettis. If this hypothesis is correct, it may be possible to breed bees that are better able to defend themselves from the mites, he says.

LT-SEM technology is an exciting new tool to help reveal the systematics and behavior of mites. It is already providing valuable new information that could be used to control them as agricultural pests or to make them more effective as biological control agents.—By **Hank Becker,** ARS.

This research is part of Crop Protection and Quarantine, an ARS National Program (#304) described on the World Wide Web at http://www.nps.ars.usda.gov/programs/cppvs.htm.

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